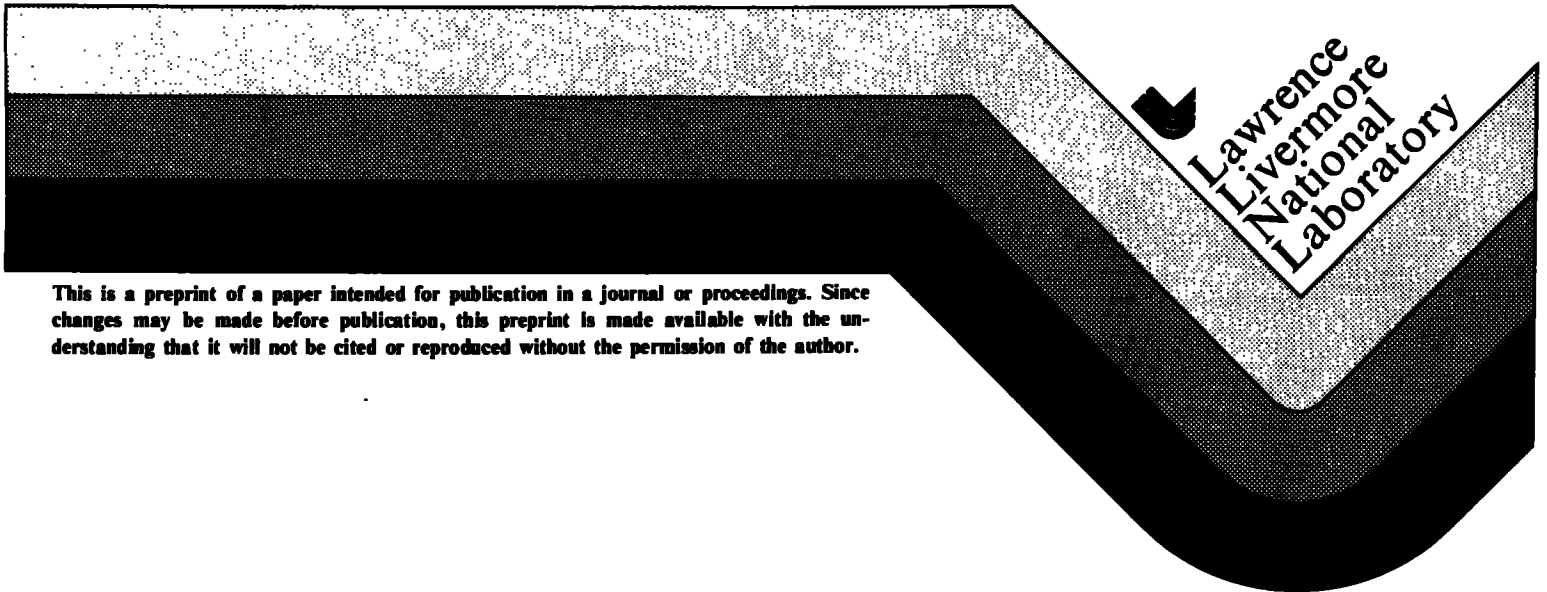# CLINICAL MEASUREMENTS USING
FIBER OPTICS AND OPTRODES

F. P. Milanovich
T. B. Hirschfeld
F. T. Wang
S. M. Klainer
D. Walt

DISCLAIMER

Clinical measurements using fiber optics and optrodes

Fred P. Milanovich
Tomas B. Hirschfeld
Francis T. Wang

Lawrence Livermore National Laboratory, University of California
Livermore, California 94550

Stanley M. Klainer

ST&E Technical Services, Inc., 20 Belinda Court, San Ramon, California 94583

and

David Walt

Department of Chemistry, Tufts University, Medford, Massachusetts 02155

Abstract

Fiber optics, optrodes, and fluorescence spectroscopy have been combined to form the new technology of remote fiber fluorimetry (RFF). Both in-vivo and in-vitro clinical measurements can be made by using this technique. The optrode, a fiber termination with preselected chemical or physical properties, is attached to the distal end of the optical fiber so that specific, in-situ measurements can be made. RFF systems for pH, blood pressure, oxygen, and carbon dioxide are being completed, and other optrodes are in the development stages.

Introduction

Low-loss optical fibers for transmitting visible light are now available. They are the outgrowth of the communications industry's need for larger bandwidth communications cables than were feasible with electrical technology. The larger cables paved the way for optical cables that are capable of transmitting infrared radiation over many kilometers with little attenuation.[1] The availability of these fibers gave rise to the new technology of fiber-optic sensors.[2] The first sensors designed to collect measurement information from remote locations, over fiber optics, relied on the fact that alterations in a specific physical property of the medium being probed would cause a predictable change in the light-transmission characteristics of the optical fiber. Acoustic waves, acceleration, strain, position, and magnetic field are some of the physical properties measured with these initial sensors. The advantages of remote optical sensors include miniaturization, long life, electromagnetic immunity, radiation resistance, geometric flexibility, ruggedness, and multiplexing. Each of these is of particular importance in clinical probes where the ultimate goal is to use safely continuous reading multiple probes for extended periods, in a variety of in-vivo and in-vitro applications, without interference from external energy sources.

The applications and versatility of remote fiber sensors were greatly increased when optrodes and fluorescence spectroscopy were added to the remote detection scheme.[3,4] Now the technique is no longer restricted to measuring physical properties that change fiber transmission but can be extended to both organic and inorganic chemical analyses and several additional physical measurements.

Remote fiber fluorimetry (RFF)

Laser-based RFF is a new analytical technique that makes it possible to measure and monitor, continuously and in real time, trace (ppm) to macro (g/l) concentrations of select chemical species as well as certain physical properties. This technique permits the determination of blood gases, electrolytes, blood pressure, etc., for clinical purposes. Fluorescence, or excitation, is stimulated by laser light of the appropriate wavelength that is focused into single-strand optical fibers. The optical fiber is coupled to the sample of interest, i.e., the blood, through the optrode. These optrodes have been developed for measuring specific chemical species or physical properties for samples in a particular matrix. The fluorescence, or stimulated light, is collected and travels back through the same fiber to an especially designed spectrometer where the particular wavelengths of the return light are spectrally sorted for analysis. The intensity of the signal in a specific wavelength channel is related to either the chemical or physical information being sought. The use of a single fiber to both transmit the

laser light and receive the fluorescence signal is of major importance for clinical sensors, since it greatly reduces probe size and minimizes optical alignment and focus problems at the sample (in the patient). Figure 1 diagrammatically shows the RFF concept.
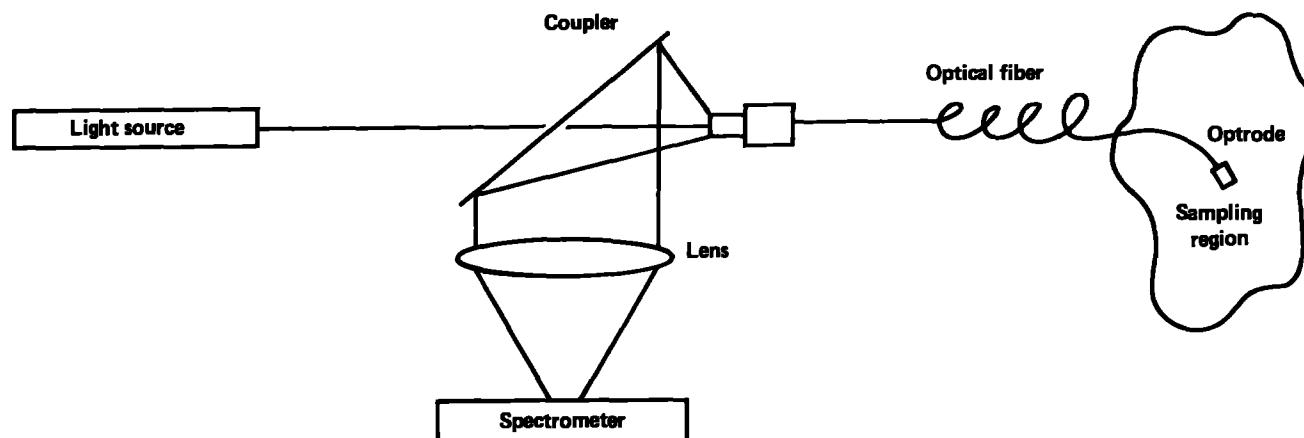
Figure 1. Schematic of the RFF concept.

Fluorescence spectroscopy

When a quantum of light impinges on a molecule, there is a probability, dependent on its wavelength, that the light will be absorbed. This process is nearly instantaneous and results in the visible and ultraviolet absorption spectra that are characteristic of many molecules. Once in the excited state, the molecule typically loses a small fraction of the excitation energy and then returns to the ground state by the emission of radiation. Consequently, the emitted light generally will be of longer wavelength than the absorbed light. Depending on the nature of the excited state, fluorescence (short-lived, $<10^{-4}$ sec) or phosphorescence (long-lived, $>10^{-4}$ sec) will occur.

Fluorescence usually is induced by visible and ultraviolet radiation. Laser sources are used normally to excite the molecule, because they provide a convenient source of high-intensity radiation, and because their monochromaticity assures that all the available energy is fully utilized. This gives maximum specificity and sensitivity.

Fluorescence is exhibited both by free atoms and molecules. It can occur in the gaseous, liquid, and solid states, although not necessarily in all three phases of the same substance. The fluorescence effect at the atomic level is well understood. Fluorescence in molecules is a more complex phenomenon, because the electronic excitation and deexcitation process may be accompanied by secondary changes in the vibrational and rotational energy of the molecule. It is molecular or ionic fluorescence that is generally applied in RFF.

Materials that fluoresce (or phosphoresce) naturally, those that can be converted to fluorescent compounds (fluorophors), and those that extinguish fluorescence can be determined quantitatively by fluorimetry. Compounds in a mixture can usually be distinguished from one another, because (a) compounds that absorb at the same wavelength usually emit radiation at different wavelengths, (b) compounds that emit at the same wavelength most often absorb at different wavelengths, and (c) transitions of different lifetimes can readily be distinguished by modern light-measuring techniques. Indeed, the advent of modern laser techniques has made fluorimetry the **most** sensitive analytical technique available.[5,6]

Remote fiber fluorescence spectrometers

There are two spectrometers being used. The first is an exceptionally versatile research unit capable of handling and measuring the output of all optrode combinations. The second is a high-sensitivity semiportable unit that is designed to handle the signal from up to four preselected optrodes. Each of these instruments contains a light source, a coupler, a grating or filters, and a data processing system and display. Figure 2a is a diagram of the laboratory RFF spectrometer. This is basically a high sensitivity Raman system with good resolution and excellent stray-light rejection.
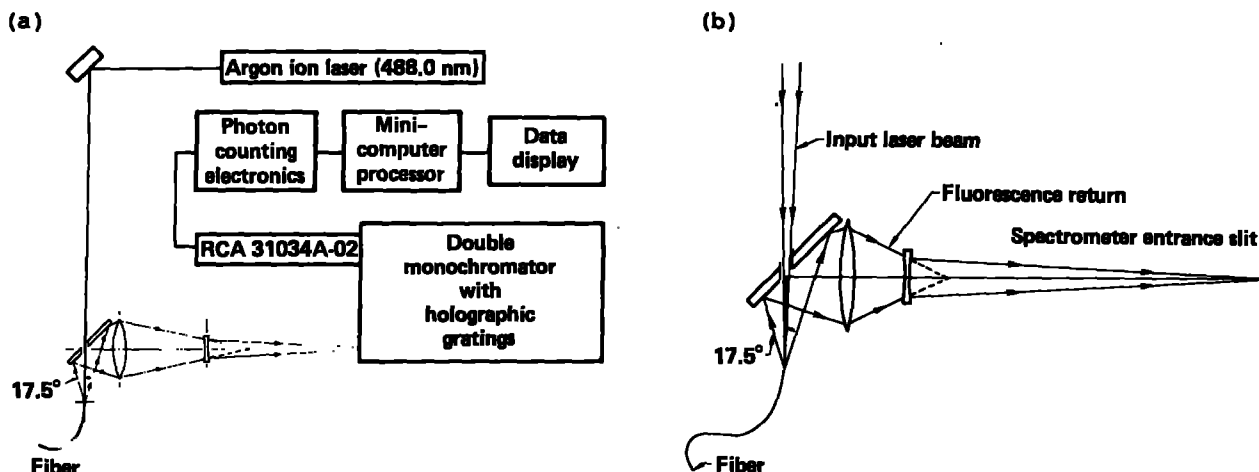
Figure 2. Schematic of (a) an RFF spectrometer showing (b) the geometric optical coupler.

The illuminator for the laboratory instrument is a high-power argon laser (Spectra Physics 165) operated with a prism wavelength selector. The power from this laser is higher than needed. Whereas the optical fibers will tolerate power levels up to a few hundred mW, the overall efficiency of the RFF system is such that with good samples or appropriate optrodes, the detector is saturated at 1 to 10-µW power levels. These lasers cannot operate at such low powers and attenuators are therefore required; first, the laser beam is expanded to reduce the power density to levels where optical filters will not be damaged, and then neutral density filters are used. In addition, line filters are used to eliminate cavity emissions at wavelengths other than the one of interest.

Coupler. A device is required to couple the laser light into the fiber and to couple the returning beam from the fiber into the spectral sorter. The coupler also makes it possible to use the same fiber for illumination and collection. This not only eliminates problems caused by fiber alignment variations and modal distortions within the fiber, but it also maximizes the overlap between the illumination and observation volumes and their subtended solid angle and thus increases sensitivity.

To use a single fiber requires that the outgoing and returning beam be separated at the fiber end. This can be done either by (a) using a simple beam splitter to separate the outgoing and returning light by their direction (inefficient), (b) using a dichroic mirror to separate the outgoing and returning beams by their wavelength (inflexible), or (c) using a mirror with a small hole in it to separate the highly focused outgoing beam from the divergent returning beam (geometric, efficient, and versatile).

Figure 2b shows how the geometric coupler accomplishes the desired separation. Here, the focused laser beam goes through the hole in the mirror and into the fiber. The returning incoherent fluorescence leaves the fiber with a higher divergence, strikes the mirror, and is turned into a collection lens followed by a focusing lens that directs the light into the spectral sorter.

The separation of concentric outgoing and returning beams can also be accomplished by using a small prism and collection lenses as shown in Figure 3. Here, the prism turns the focused laser beams into the fiber while the divergent returning light is collected by the lens system and focused into the spectral sorter. Because of its small size, the prism causes essentially no obscuration and thus no degradation of signal intensity.

Optical fiber. Fibers used in clinical systems are Corning glass on glass Coreguide fibers, which have a 100-µm core and a 140-µm overall diameter. Spectral examination of these fibers using 10 µW of 488-nm irradiation showed them to be essentially free of interfering spectral components. (The Raman transitions of these fibers are of no concern due to the low laser power and short fiber lengths used in biomedical applications.)

Considerable caution was exercised when installing the coupler to the fibers, even though transmission through the fibers was as expected. Because of the small fiber size, alignment is critical; losses were observed when the fiber was not properly centered in the coupler. In addition, the fiber, in the coupler, must be properly polished to avoid end damage during connections. Extreme cleanliness is required when terminating the fiber to avoid interfering fluorescence from the sheath near the inlet of the fiber. To avoid sheath excitation the illuminating beam and collection field-of-view must be properly vignetted.
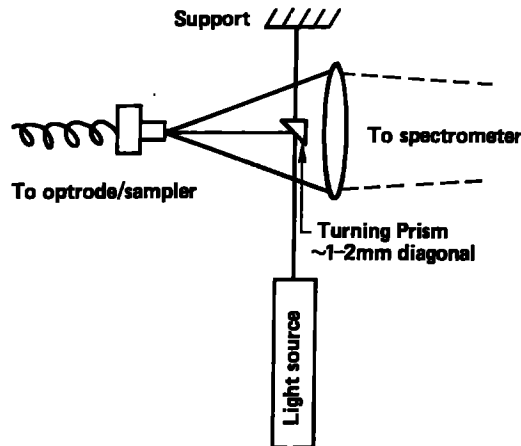
Figure 3. Schematic of prism coupler.

Spectral sorter. The spectral sorter is a 3/4-m double holographic grating monochromator (Spex 1401). This instrument uses f/8 illumination from a photographic objective and is scanned by a digital stepping scanner. The monochromator is coupled to a GaAs cathode quantum-counting end-on photomultiplier (RCA C31034A) connected to a high-voltage power supply and a discriminator counter (Ortec 9302). These are connected to a recorder (Hewlett-Packard 7004B) and to the data system. The photomultiplier is operated in a Products for Research thermoelectrically cooled mount and housing.

Data system. The output of the counter is fed into a system voltmeter (Hewlett Packard 3435), which is controlled by a timer that digitizes the ratemeter output and feeds it through an HP1B connection to a computer (LSI-11/23) with 64K of memory and two 8-in. 256KB diskettes. The system is set up so that the data from the computer can be fed to a graphics processor (Tektronics 4051) with a hard-copy unit or plotted out directly on an XY plotter (Tektronix 4662).

Semiportable RFF spectrometer. Figure 4 is a diagram of the semiportable RFF spectrometer. This is basically a very high-sensitivity dedicated unit with essentially no capacity to handle undefined optrodes. The arrangement shown in Figure 4 is a 2-channel system. Two optrodes or a single optrode with a guard channel can be handled using this design.
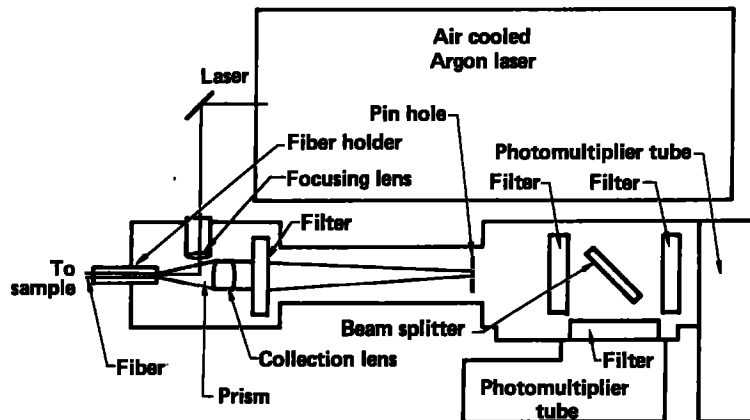

Figure 4. Schematic of semiportable RFF spectrometer.

The illuminator for the semiportable instrument is a low-power air-cooled argon laser (Spectra Physics 51). This unit has a 20-mW power output at 488 nm. As in the case of the laboratory system, the beam is attenuated with neutral density filters to reduce the power to the 1-μW range, and a line filter is used to assure that only the 488-nm illumination excites the sample.

Coupler. In this instrument, the prism coupler, as previously described (Figure 3), is used. Here, a lens focuses the laser beam on the prism, which turns the beam and focuses it on the fiber. The input beam is smaller than the fiber and thus underfills it. The return fluorescence is collected and conditioned so that it is perpendicular to the spectral sorter.

Spectral sorter. To guarantee maximum sensitivity and specificity, spectral sorting is accomplished in three phases. First, the exiting beam goes through an interference filter, is focused through a pinhole, is expanded and conditioned, and then it goes through a second interference filter. The purpose of this part of the optical train is to remove any stray or reflected 488-nm light. Inasmuch as these filters will be exposed to 488-nm illumination, they must be carefully selected to assure that they do not fluoresce. Second, the beam, which now contains only the wavelengths of the emitted light, is partially separated by using a beam splitter. In the present instrument, a band-pass filter, which has angular wavelength dependence, is used. Proper angle selection (with relation to the oncoming fluorescent light) causes the fluorescence below a certain wavelength to be reflected while the rest of the light is transmitted. Placing two color filters in front of the photomultiplier tubes achieves final spectral sorting.

Data system. Instrument functions and signal processing are managed by a hard-wired computer system. The present unit provides for photon counting in each data channel, background correction, and ratioing of the data from channel to channel. Photomultiplier high voltage and overload are monitored. Provisions are made for expanding the computational capability to include self-calibration, automatic exposure control, preselected measurement sequences, direct reading in clinical terminology, self-regulating gain control, and instrument housekeeping functions. The present system has none of these features and gain is selected using switches.

Optrodes

The key component of the RFF system is the optrode, many types of which have been described in previous papers.[4,7,8] The optrode is a special termination of the distal end of the fiber, which aids in the increase, decrease, or initiation of the fluorescence when the exciting light interacts with the parameter or species to be measured. Optrodes can either be physical or chemical. Physical optrodes are mechanical or direct measuring while chemical optrodes may use reagents, which have been immobilized or are in reservoirs and membranes. The clinical optrodes, however, have much more stringent specifications and applications; new technologies have had to be developed to meet these needs.

Physical optrodes. Figure 5 shows the design for a pressure optrode. Here, a fluorescence (or reflective) surface is held in place, at the terminal end of an optical fiber, by a flexible bellows. The spectrometer measures the intensity of the fluorescence from an excitation source whose output power is stabilized or accurately controlled. The fluorescence intensity changes with the position of the bellows, which in turn varies with the surrounding pressure. Figure 6 shows a calibration of a pressure optrode. A miniaturized (~250-μm diam) version of this optrode has been applied to blood pressure measurements. This optrode not only gives a complete frequency spectrum of the pressure response, but it also gives the spectrum at frequencies where existing devices tend to resonate.

Development is underway to extend extant temperature optrodes to physiological use. Temperature-dependent narrow-line fluorescence emissions have been observed for some lanthanides and transition metals that have been sequestered on glass or a crystal-like medium. Of particular interest is the 694.3-nm emission of ruby. This transition is a doublet, and the ratio of the intensities of the two peaks is a strict function of temperature. Ruby is available in sizes compatible with most optical fiber diameters or, if very small optrodes are needed, the tip of a sapphire fiber can be turned into ruby by heat treatment in a chromium atmosphere.[9] Europium- and neodymium-doped glasses have also been used. The present search is for a dopant that has a steep temperature slope between 95 and 110°F.

Chemical optrodes. Optrodes that are sensitive to selected chemical species also exist. A basic type of optrode used for these measurements is shown schematically in Figure 7. In this case immobilization is accomplished by trapping the reactant in a porous polymer (or by using other standard immobilization techniques[10,11]).

This optrode design has the advantage of being amenable to mixed chemical systems, thus extending possible compound coverage by providing a matrix for complex chemical reactions, if needed. This can be achieved by immobilizing the various reagents in different polymer layers and allowing them to interact, through diffusion, for example, in a controlled manner. The reagents trapped in the polymer are available to react with the species of interest causing a fluorescence response. Membranes can be used in conjunction with this
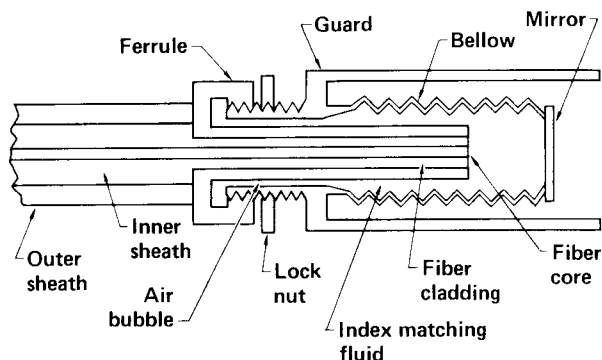
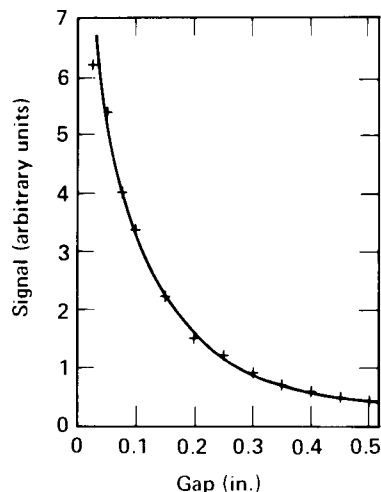Figure 5. Schematic of bellows pressure optrode.

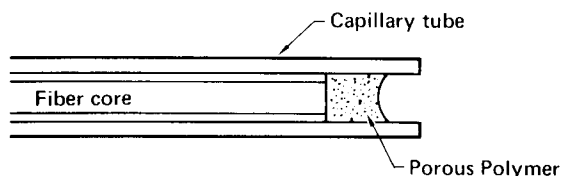Figure 6. Calibration of a pressure optrode.

Figure 7. Schematic of a chemical optrode type.

optrode to either improve specificity by rejecting interfering compounds, or increase optrode life by containing the reagents in the capillary. The big drawback of this design is size. The use of the capillary probably means an optrode diameter >200 μm. Figure 8 is a photograph of this type of optrode.
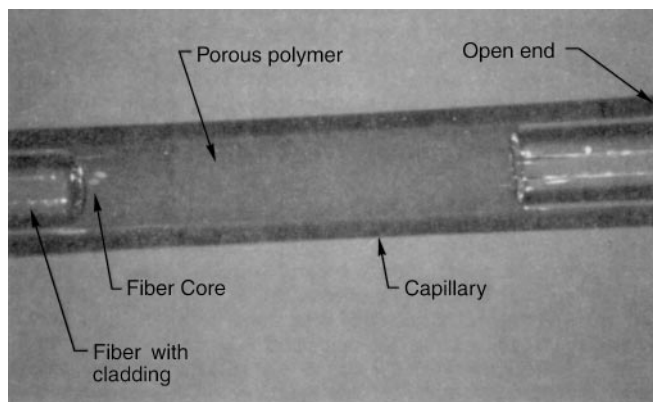

Figure 8. Photo of a porous polymer optrode.

The pH optrode has been constructed using fluorescein as the indicator reagent. To make this compound responsive in the physiological range (pH 6.8 to 7.8) the fluorescein structure had to be modified. Response times are less than 3 min. As can be seen from Figure 9, there is a steep dependence of fluorescence intensity on pH . Final results indicate that 0.01 to 0.02 pH units should be resolvable. Figure 10 shows the response of this optrode to pH. This curve also gives an indication of the repeatability of the measurements.

Conclusions

RFF represents a major technical advance in in-vivo measurements. The small size, fast response, long life, and projected low cost make optrodes an ideal clinical tool. Several
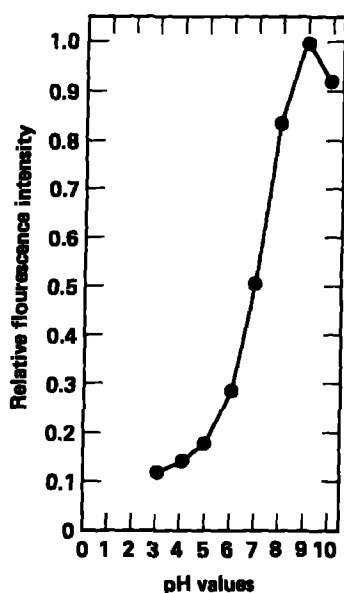
Figure 9. pH response of fluorescein
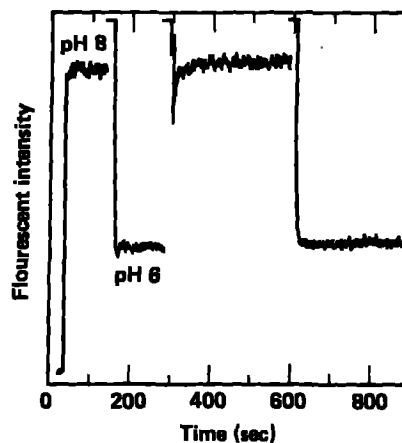fluourescence intensity.

Figure 10. Strip chart of pH
optrode, pH 8-6-8-6.

optrodes in advance stages of development include blood pressure, pH, oxygen, and carbon dioxide. Other optrodes in development include potassium, sodium, and chloride, which are the precursors to an in-vivo electrolyte measuring system. In-vitro measurements are also possible using optrodes, but they do not take full advantage of the capabilities of the methodology.

References

1. Gunderson, L. C. and Keck, D. B., "Where Light Outperforms Electrons," Technol. Rev., May/June, pp. 34-44. 1983.
2. Vali, V. and Shorthill, R. W., "Fibre Ring Interferometer," Appl. Opt., Vol. 15, pp. 1099-1100. 1976.
3. Hirschfeld, T., Haugen, G. R., Johnson, D. C., and Hrubesh, L. W., "Remote Techniques Based on Long-distance Fiber Optics," 179th National Meeting of the American Chemical Society, Houston, Texas, 1980.
4. Hirschfeld, T., Deaton, T., Milanovich, F., and Klainer, S., "Feasibility of Using Fiber Optics for Measuring Groundwater Contaminants," Opt. Eng., Vol. 22(5), pp. 527-531. 1983.
5. Richardson, J. H., "Applications of Lasers in Analytical Molecular Fluorescence Spectroscopy," Modern Fluorescence Spectroscopy, E. H. Wehry, Ed., Plenum, New York, Vol. 4,. 1981.
6. Perry, D. L., Klainer, S. M., Bowman, H. R., Milanovich, F. P., Hirschfeld, T., and Miller, S., "Detection of Ultratrace Levels of Uranium in Aqueous Samples by Laser-induced Fluorescence Spectrometry," Anal. Chem., Vol. 53(7), pp. 1048-1050. 1981.
7. Deaton, T., "Instrumentation and Methodology for Remote Fiber Fluorimetry," Ph.D. Thesis, University of California at Davis, Davis, California. 1984.
8. Milanovich, F. P. and Hirschfeld, T., "Process Product and Waste Stream Monitoring with Fiber Optics," Advances in Instrumentation, Vol. 38, Part 1, pp. 407-418. 1983.
9. Hirschfeld, T., Wang, F., and Milanovich, F. P., "Ultra-fast Thermometers Based on Fiber Optics," Meeting of the Federation of Analytical Chemistry and Spectroscopy Societies, Philadelphia, Pennsylvania. 1982.
10. Mosbach, K., Ed., "Methods in Enzymology. XLIV. Immobilized Enzymes," Academic, New York, XLIV. 1976.
11. Johnson, J. C., "Immobilized Enzymes, Preparation and Engineering, Recent Advances," Noyes Data Corporation, New Jersey. 1979.